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Electromagnetic field exposure-induced depression features could be alleviated by heat acclimation based on remodeling the gut microbiota

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ABSTRACT

Background: Electromagnetic pollution cannot be ignored. Long-term low-dose electromagnetic field (EMF) exposure can cause central nervous system dysfunction without effective prevention.

Materials/Methods: Male C57BL/6J mice (6–8 weeks, 17–20 g) were used in this study. Depression-like and anxiety-like behaviors detected by behavioral experiments were compared among different treatments. 16S rRNA gene sequencing and non-targeted liquid chromatography-mass spectrometry (LC-MS) metabolomics were used to explore the relationship between EMF exposure and heat acclimation (HA) effects on gut microbes and serum metabolites.

Results: Both EMF and HA regulated the proportions of *p_Firmicutes* and *p_Bacteroidota*. EMF exposure caused the proportions of 6 kinds of bacteria, such as *g_Butyricicoccus* and *g_Anaerotruncus,* to change significantly ($p < 0.05$). HA restored the balance of gut microbes that was affected by EMF exposure and the proportion of probiotics (*g_Lactobacillus*) increased significantly ($p < 0.01$). Serum metabolite analysis suggested that HA alleviated the disturbance of serum metabolites (such as cholesterol and D-mannose) induced by EMF exposure. Both the metabolic KEGG pathways and PICRUSt functional analysis demonstrated that tryptophan metabolism, pyrimidine metabolism and amino acid biosynthesis were involved.

Conclusions: EMF exposure not only led to depression-like neurobehavioral disorders, but also to gut microbiota imbalance. HA alleviated the depression features caused by EMF exposure. Based on the analysis of gut microbiota associated with serum metabolites, we speculated that gut microbiota might play a vital role in the cross-tolerance provided by HA.

1. Introduction

Electromagnetic fields (EMFs) are widely used in communication, smelting, medicine and other industries. The impact of electromagnetic pollution on health has attracted public attention. EMF regulation (2016) clearly noted that long-term low-dose electromagnetic radiation exposure can lead to central nervous system dysfunction (Van, 2000; Wijngaarden, 2000). EMF exposure can cause neurobehavioral disorders, including circadian imbalance, headache, fatigue, depression and anxiety. However, it is difficult to verify the conclusions of epidemiological studies on the mechanism of nerve injury induced by EMF exposure, especially low-dose EMF exposure. Some of the obtained results have even been contradictory. One reason might be that researchers analyzed the bioeffects of EMFs based on the levels in cells or tissues and neglect to look at the experimental animals as a whole.

The gut microbiota is a complex microecological system that colonizes the intestine. Studies have shown that gut microbes are closely related to health (Wang et al., 2012). They regulate host energy homeostasis, material metabolism, blood glucose balance and the immune response. More importantly, gut microbes also play an essential role in maintaining brain physiological function, participating in neuropsychiatric behavior, brain development, aging and neurodegenerative processes (Sun et al., 2020). Chronic environmental stimulation could lead to anxiety, depression and other mental and behavioral disorders based on gut microbes (Neufeld et al., 2014; Sharon et al., 2016). As a physical environmental factor, EMFs might also lead to central nervous

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system disorders by affecting gut microbes. Currently, there are few reports about the bioeffects of gut microbes under EMF exposure. In 2020, Yee. et al. found that the gut microbiome ratio of *Firmicutes/Bacteroidetes* decreased with pulsing electromagnetic field (PEMF) exposure (Yee et al., 2020). Therefore, EMF exposure could be able to affect the gut microbiota composition.

Heat acclimation (HA) refers to a series of adaptive physiological responses to long-term repeated exposure to moderately high environmental temperatures. Studies have confirmed that HA could not only alleviate central nervous system injury caused by heatstroke (Yi et al., 2017),but also enhance the protective effect of other stimuli besides high temperature (such as hypoxia, ischemia and reperfusion injury) on injury (Umschweif et al., 2013). This cross reinforcement is called "cross tolerance". Our previous study also revealed that HA could regulate microglial inflammatory activation with EMF exposure (Genlin et al., 2020). Moreover, long-term exposure to high environmental temperature has been shown to affect the gut microbiome in mice (Yang et al., 2021). According to the "cross tolerance" of HA, we hypothesize that HA might play a protective role in disorders of the central nervous system against EMF exposure by remodeling the gut microbiota.

Our study used a mouse model of intermittent long-term EMF exposure to explore the adverse biological effects of EMFs. Additionally, a mouse model of HA was used. Central nervous system dysfunction was confirmed by animal behavior experiments. 16S rRNA gene sequencing was used to explore variations in the gut microbiome and combined with non-targeted liquid chromatography–mass spectrometry (LC-MS) metabolomics to elucidate the potential mechanism by which HA alleviates the depression features caused by EMF exposure.

2. Methodology and materials

2.1. Experimental animal model

2.1.1. Heat acclimation model

All experimental animals were[1] 8-week-old male nice purchased from the Animal Centre of Army Medical University (Chongqing, China). The breeding conditions for mice were 28 degrees Celsius, 50% humidity and a 12:12 h light:dark cycle with food and water provided ad libitum. The mice were randomly divided into four groups: the control group (Control), electromagnetic field exposure group (EMF), electromagnetic field exposure with heat acclimation group (EMF+HA) and heat acclimation group (HA). All animal model included two stages. The first stage was 28 days and the mice in the control group (Control) and electromagnetic field exposure group (EMF) were housed at ambient temperature (28 ± 0.5 °C), while the heat acclimation group (HA) and electromagnetic field exposure with heat acclimation group (EMF+HA) were housed at 35 ± 0.5 °C with 60 ± 5% humidity (Fig. 1A). During this period, the basal metabolism and heart rate of mice in HA group were lower and the heat resistance was better.

2.1.2. Electromagnetic field (EMF) exposure model

To keep the temperature and humidity constant in the EMF exposure environment, pulsed electromagnetic field (2450 MHz, 2 μs/pulse, 500 pulses/s) were employed. Experimental animals were deposited in a special plexiglass cage that allowed for transmission of electromagnetic waves and the long axis of the mouse body was perpendicular to the long axis of the electromagnetic field-emitting antenna. Whole bodies of mice were exposed to EMF (specific absorption rate, SAR: 2.5 W/kg) for 4 h a day for 5 weeks. After exposure, the EMF+HA group was returned to an artificial climate simulation cabin with continued HA (temperature: 35 ± 0.5 °C, humidity: 60 ± 5%), while the EMF group was returned to ambient temperature (temperature: 28 ± 0.5 °C, humidity: 50 ± 5% humidity). Eliminating the environmental interference caused by EMF exposure, the HA group was also placed in a plexiglass cage with sham EMF exposure for 4 h a day for 5 weeks. When animals underwent EMF exposure, the environmental temperature was 28 ± 0.5 °C, and the humidity was 50 ± 5% (Fig. 1B). All animal laboratories involved in this study not only strictly followed the recommendations in the National Institutes of Health's Laboratory Animal Care and Use Guidelines but were also approved by the Laboratory Animal Welfare and Ethics Committee of Army Medical University.

2.2. Methods

2.2.1. Sample collection and processing

After behavioral testing, mice were anesthetized, and blood was collected from the eyeballs. Serum samples were immediately frozen in liquid nitrogen and then stored at − 80 °C. Fresh feces from each animal were collected at the end of the experiment. Each mouse was placed separately in a metabolic cage. The samples were immediately frozen in liquid nitrogen and then stored at − 80 °C until DNA extraction.

2.2.2. Behavioral experiments

All assays to monitor behavior commenced 12 h after the end of EMF exposure. The mice were kept in the same room in which the experiments were to be performed for a minimum of 30 min before each test. Furthermore, the less stressful test (open-field test) was conducted before the more stressful tests (elevated plus-maze, light–dark transitions, forced swim test, tail suspension test).

2.2.3. Open-field test

The open-field test allowed for the assessment of behavior and locomotor activity by recording the free movements of the mice. The open field maze consisted of a square chamber (40 cm (length) × 40 cm (width) × 25 cm (height)) made of nonporous plastic placed in a quiet environment free of clues. The mouse was placed in the middle of the maze and could freely move for 5 min. Locomotion was automatically recorded using an EthoVision XT15 (Noldus, Wageningen, The Netherlands) video tracking system attached to a pole above the field. During the test, the total distance and central square duration were all obtained from the tracking system with a video camera mounted above the apparatus. After each subsequent test, the entire apparatus was cleaned with 75% ethanol.

2.2.4. Elevated plus-maze test

The apparatus consisted of two sets of arms: two open arms and two closed arms. The dimensions of both the open and closed arms were 30 cm × 6 cm × 15 cm. Each of the arms was associated with a common central platform having dimensions of 6 cm × 6 cm. Individual mice were placed on the central platform with their heads pointed toward the open arm of the apparatus. Subsequently, the ratios of open-arm time to total arm time and open-arm frequency to total arm frequency were calculated for a period of 10 min. Prior to each test, the platform was cleaned using 75% ethanol.

2.2.5. Light/dark transition test

The apparatus used comprised a light side and a dark side having identical dimensions of 20 cm × 15 cm × 25 cm. The experiments were initiated by placing mice on the light side of the chamber first. After 10 min of recording, the time spent in the dark side of the chamber and the total number of transitions were recorded. Prior to each test, the platform was cleaned using 75% ethanol.

2.2.6. Tail suspension test

This test was carried out by suspending each mouse by its tail with the help of bands from a hook placed 50 cm above floor level. The animals being tested were isolated from each other both acoustically and visually. The hanging was continued for 6 min. The entire period was recorded by a camera and the immobility times were monitored by an

[1] 6-8-week-old male nice.

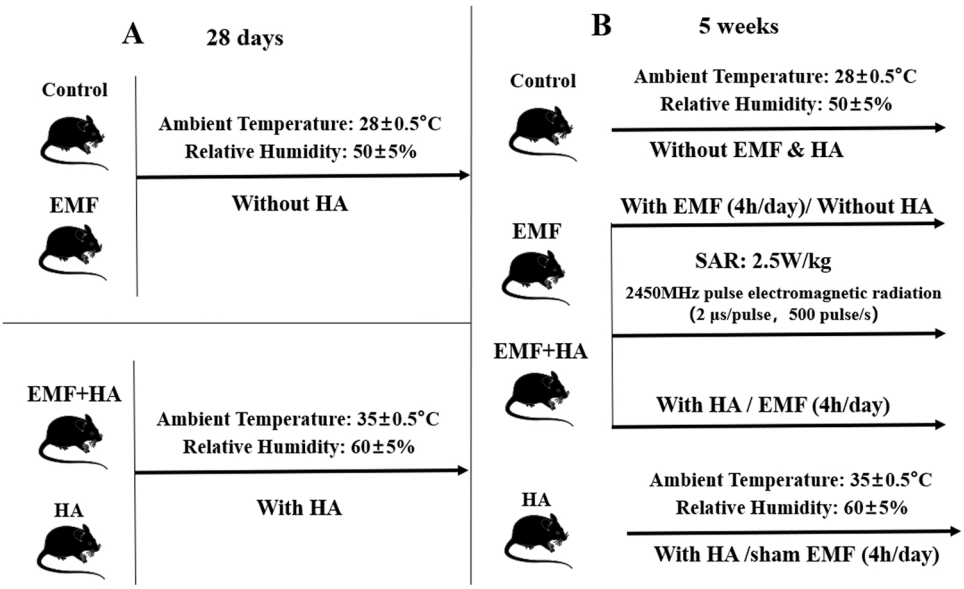


Fig. 1. Research design. A. Mice were randomly divided into four groups: the control group (Control), electromagnetic field exposure group (EMF), electromagnetic field exposure with heat acclimation group (EMF+HA) and heat acclimation group (HA). The process of heat acclimation was 28 days. Mice in the control group (Control) and electromagnetic field exposure group (EMF) were housed at ambient temperature (28 ± 0.5 °C), while heat acclimation group (HA) and electromagnetic field exposure with heat acclimation group (EMF+HA) at the temperature of (35 ± 0.5 °C) with 60 ± 5% humidity reared. B. After the 28 days HA, 5-week EMF exposure model was conducted. The pulsed electromagnetic fields (2450 MHz, 2 μs/pulse, 500 pulses/s) were employed. Experimental animals deposited in a special plexiglass cage that could transmit electromagnetic waves and the long axis of the mouse body should be perpendicular to the long axis of the EMF emitting antenna. Whole body of mice were exposed to EMF (specific absorption rate, SAR: 2.5 W/kg) for 4 h a day with 5 weeks. After exposure, EMF+HA group were returned to artificial climate simulation cabin continuing heat acclimation (temperature: 35 ± 0.5 °C, humidity: 60 ± 5%), while EMF group were returned to ambient temperature (temperature: 28 ± 0.5 °C, humidity: 50 ± 5% humidity). Eliminating the environmental interference caused by the EMF exposure, HA group was also placed in plexiglass cage with sham EMF exposure for 4 h a day with 5 weeks. When animals underwent EMF exposure, the environmental temperature was 28 ± 0.5 °C and humidity was 50 ± 5%.

uninformed observer.

2.2.7. Forced swimming test

A glass cylinder with a diameter of 10 cm and height of 20 cm was filled with water up to a level of 10 cm. Temperature was maintained at 23 ± 2 °C. The mice were placed in the middle of the given cylinder and allowed to swim for 6 min. Swimming was monitored for the entire period. However, only the 4-min period toward the end was taken into consideration for result analysis. The duration of immobility was measured by an observer unaware of the groups.

2.3. 16S rRNA gene sequencing & analysis

DNA was isolated from fecal pellets using an EZNA stool DNA kit according to the instructions of the manufacturer (Omega Biotek, Inc., Norcross, GA). DNA was amplified using universal primers (iTRU-A 515F and iTRU-1 806R) to target the V4 regions of bacterial 16S rRNA. Individual samples were barcoded, pooled to construct the sequencing library and then sequenced (Illumina MiSeq) to generate paired-end 250 × 250 reads. Analysis of 16S rRNA sequencing data was performed by Shanghai Majorbio Biopharm Technology Co., Ltd.

2.4. Serum metabolomics profiling

LC-MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher, Waltham, MA, USA) coupled with an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher, Waltham, MA, USA). For the serum samples, mobile phase A was water mixed with 0.1% formic acid, while mobile phase B was 70% isopropanol and 30% acetonitrile containing 0.1% formic acid. The serum samples were eluted under gradient conditions at a flow rate of 400 μl/min with 1% B, which was held for 1 min and then ramped up from 1% to 40% B for 2 min, from 40% to 75% B for 5 min, from 75% to 85% B for 4 min and from 85% to 99% B for 6 min, held at 99% B over 4 min and then returned to 1% B for 3 min. The volume of the sample injected onto the column was 1000 μl.

3. Results

3.1. EMF exposure did not cause anxiety-like behavior in mice

We used the open-field test (OFT), elevated plus maze (EPM) and light/dark transition (LDT) test to determine whether there was anxiety-like behavior in mice. The results of the open-field test for distance covered in 5 min travel time by the different groups of animals were analyzed (Fig. 2A). There was no significant effect of the total distance traveled ($F = 0.000$, $p > 0.05$, Fig. 2B) observed. Moreover, the central square duration ($F = 0.132$, $p > 0.05$, Fig. 2C) in each group was not significantly different. Time spent by the animals on the open arm of the EPM was considered inversely related to anxiety. Mice in each group spent the same time in the open arms ($F = 0.880$, $p > 0.05$) and had an equal percentage of entries in the open arms ($F = 1.342$, $p > 0.05$, Fig. 3A). The light/dark transition (LDT) revealed no significant differences in time in the dark ($F = 0.008$, $p > 0.05$) with no significant differences in transitions ($F = 0.165$, $p > 0.05$, Fig. 3B) among the four groups in our study. These results did not indicate anxiolytic-like behavior.

3.2. EMF exposure caused depression-like behavior, while HA alleviated it

The tail suspension test (TST) and forced swimming test (FST) were used to assess depressive-like behaviors in mice. Enhanced immobility in both the TST and FST was considered a measure of behavioral despair. TST results revealed significant differences in mobility among the four groups of mice ($F = 23.484$, $p < 0.001$). Data analyses proved that mice with EMF exposure demonstrated significantly more immobility (49.071 s (31.34–66.79 s)) during the TST than control mice ($p < 0.001$). Administration of HA significantly decreased the immobility period (−36.66 s (−55.06 s to −18.27 s)) compared to that of the EMF group ($p < 0.001$, Fig. 3C). To confirm the TST results, we analyzed immobility duration using the FST and found that there were significant differences between the four groups in the FST ($F = 80.254$, $p < 0.001$). Analyses showed that mice with EMF exposure remained immobile for a significantly longer period of time than control mice ($p < 0.001$).

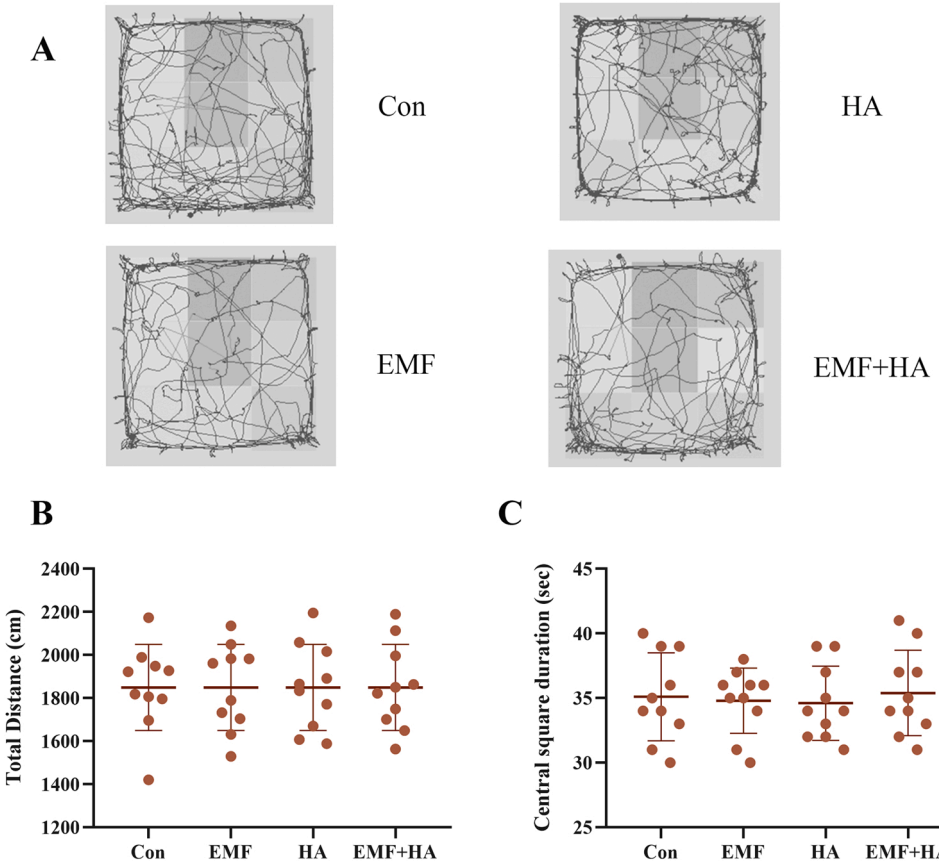


Fig. 2. Effects of HA on mice with EMF exposure in the open-field test (OFT). A. The open-field test for distance covered in 5 min travel time by the different groups. The locomotion track in the four groups. B. The total distance of each group had no significant differences ($F = 0.000$, $p > 0.05$). C. The central square duration in each group had no significant difference ($F = 0.132$, $p > 0.05$). Data represented as mean ± SEM ($n = 10$).

Administration of HA markedly reduced the duration of immobility in the FST in the EMF+HA group ($p < 0.001$, Fig. 3D). Therefore, HA ameliorated depression-like mental behavior disorder caused by chronic exposure to electromagnetic radiation.

3.3. EMF exposure causes disorder of the gut microbiome and increases depression-related metabolites

3.3.1. EMF induced changes in the gut microbiota

The OTU sequence number of each sample was normalized according to the minimum quantity of OTU sequences (CON_1:35149). Alpha diversity was determined by the analysis of species diversity in a single sample, including community diversity and richness. Shannon and Simpson indices were used to describe community diversity. There was no significant difference in community diversity between the control group and EMF group ($p > 0.05$, Wilcoxon sum test). ACE and Chao 1 indices were used to describe community richness. There was no significant difference in community richness between the control group and EMF group ($p > 0.05$, Wilcoxon sum test). Alpha diversity showed no significant difference between the control group and EMF group. Nonmetric multidimensional scaling (NMDS), based on Bray-Curtis distance, showed that EMF subjects were clearly separated from control subjects ($p = 0.003$, stress = 0.074, R = 0.48, ANOSIM for Bray-Curtis distances).

At the phylum level, the dominant bacteria in the control group were *p_Bacteroidota* (accounting for 77.5%), followed by *p_Firmicutes* (accounting for 22.4%). The dominant bacteria in the EMF group were *p_Bacteroidota* (accounting for 56.8%), followed by *p_Firmicutes* (accounting for 43.0%). The genus level analysis showed that *f_Muribaculaceae* and *g_Lactobacillus* were significantly decreased, while *g_Lachnospiraceae_NK4A136* and *g_Bacteroides* were significantly increased in EMF subjects (Fig. 4D, E).

Differentially abundant taxa were further confirmed by LEfSe analysis (Segata et al., 2011). The results are shown in Fig. 5B, and multiple genera were present in significantly different abundances in the gut microbiome between the EMF and control groups. These included *g_Butyricicoccus* (LDA = 3.65, $p = 0.032$), *g_Lachnospiraceae_NK4A136_group* (LDA = 4.94, $p = 0.022$), *g_Anaerotruncus* (LDA = 3.84, $p = 0.032$), *g_Lachnospiraceae_UCG-006* (LDA = 3.84, $p = 0.032$), *g_Bilophila* (LDA = 3.83, $p = 0.045$), and *g_Tuzzerella* (LDA = 3.80, $p = 0.022$, Fig. 5B).

3.3.2. EMF induced disturbance in the metabolite profiles of serum samples

Since 16S rRNA sequencing revealed that the gut microbiome had significantly changed with EMF exposure, we further examined whether the serum metabolomes were perturbed with EMF exposure using untargeted LC-MS in both positive ion (ES+) and negative ion (ES-) modes. To identify the differential metabolites associated with EMF exposure, we calculated fold changes (FCs), p values and PLS-DA variable importance in the projection (VIP) scores for all metabolic features in EMF vs. control. In total, 22 significant metabolic features ($FC \geq 2$ or ≤ 0.5, $p \leq 0.05$, and $VIP > 1$) were found (Table 1), including cholesterol, ketoleucine, formylanthranilic acid, maleic acid, D-fructose, fumaric acid, phenylacetylglycine, dodecanoic acid, 4-hydroxycinnamic acid and sedoheptulose 7-phosphate. Except for fumaric acid, which was significantly downregulated in the EMF group, the other 9 different metabolites were significantly upregulated with EMF exposure (Table 1). Fig. 6A presents 20 differential metabolites.

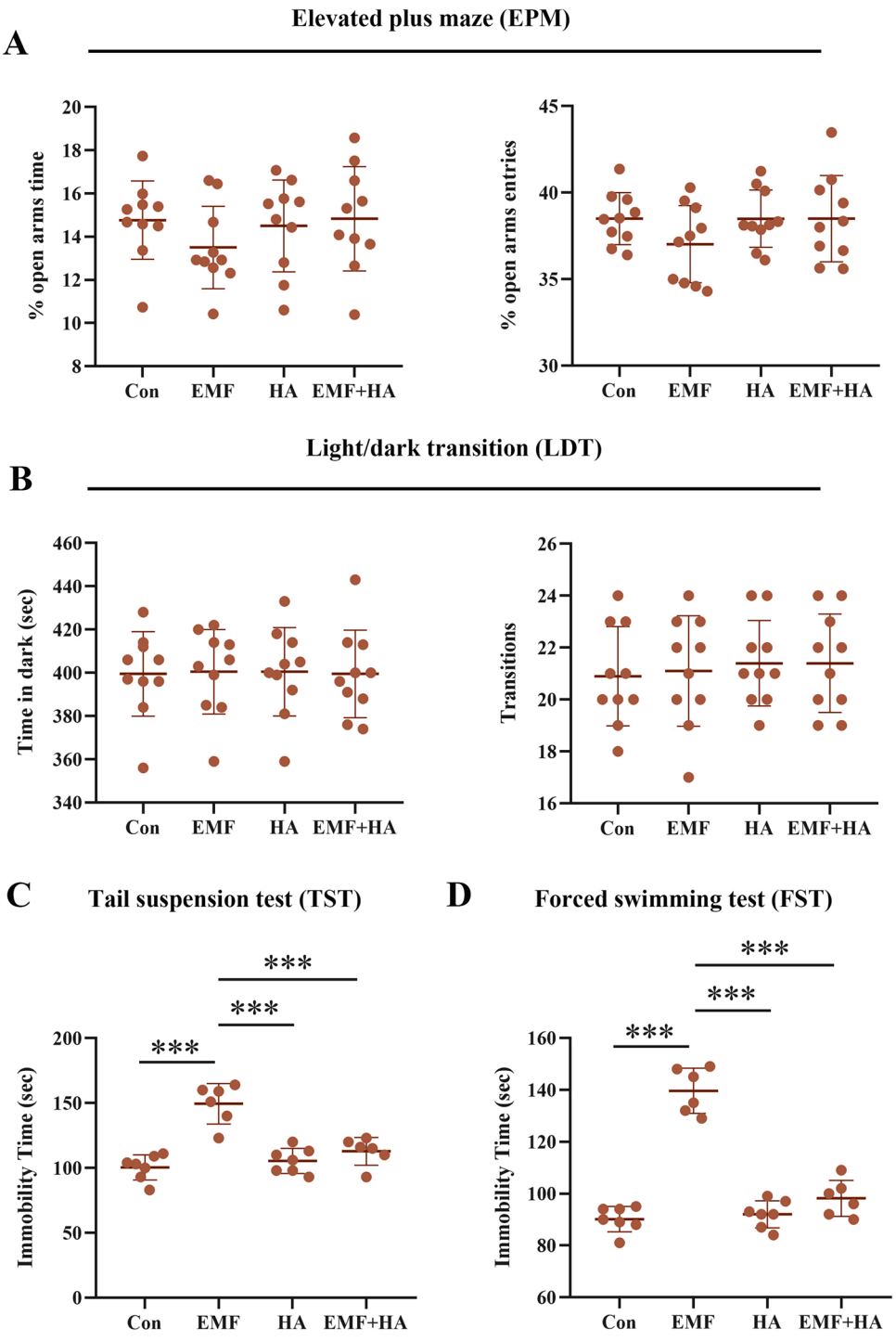


Fig. 3. Effects of HA on mice with EMF exposure in the behavioral experiments. A. Behavior shown by the four groups of animals in the elevated plus-maze test (EPM) test. Mice in each group spent same time in the open arms and had equal percentage of entries in the open arms. B. Behavior shown by the four groups of mice in the light/dark transition (LDT) test. There were no significant differences in time in the dark with no significant differences in transitions among the four groups. C. The mice with EMF exposure exhibited more immobility time on the TST, and this effect could be reversed by treatment with HA. D. The immobility time of mice with EMF exposure was more on the FST and this effect could be reversed by treatment with HA. Data are represented as mean ± SEM (n = 10). ***, $p < 0.001$.

3.4. HA remodeling of gut microbiota disorders caused by EMF exposure

3.4.1. Community composition analysis of the gut microbiota

Phylum level. The predominant bacterial phyla in all the samples were *p_Firmicutes* and *p_Bacteroidota*. Fig. 4A shows the percent abundance range for *p_Firmicutes* and *p_Bacteroidota* for various experimental groups. EMF exposure caused a significant reduction in *p_Bacteroidota*. Treatment with HA inhibited these changes. NMDS based on Bray-Curtis distances showed that different groups were clearly separated (p = 0.001, stress = 0.173, R = 0.563, ANOSIM for Bray-Curtis distances, Fig. 4B). To explore the relationship between the gut microbiota community composition and environmental characteristics, redundancy analysis (RDA) was used. RDA revealed that the gut microbiota community composition was formed by environmental characteristics, including temperature (T) and EMF exposure (E). Compared with temperature (T, $R^2 = 0.11$, $p = 0.267$), the effect of the EMF (E, $R^2 = 0.44$, $p = 0.002$) was more significant (Fig. 4C).

3.4.2. Community composition differences in the gut microbiota

The genus-level analysis showed that HA could balance the community composition under exposure to the EMF (Fig. 4E). *g_Lactobacillus* (a major probiotic, $p = 0.01842$, *corrected p-value* = 0.1413), *g_norank_f_norank_o_Clostridia_UCG-014* ($p = 0.003405$, *corrected p-value* = 0.0893), *g_norank_f_Oscillospiraceae* ($p = 0.02681$, *corrected p-value* =

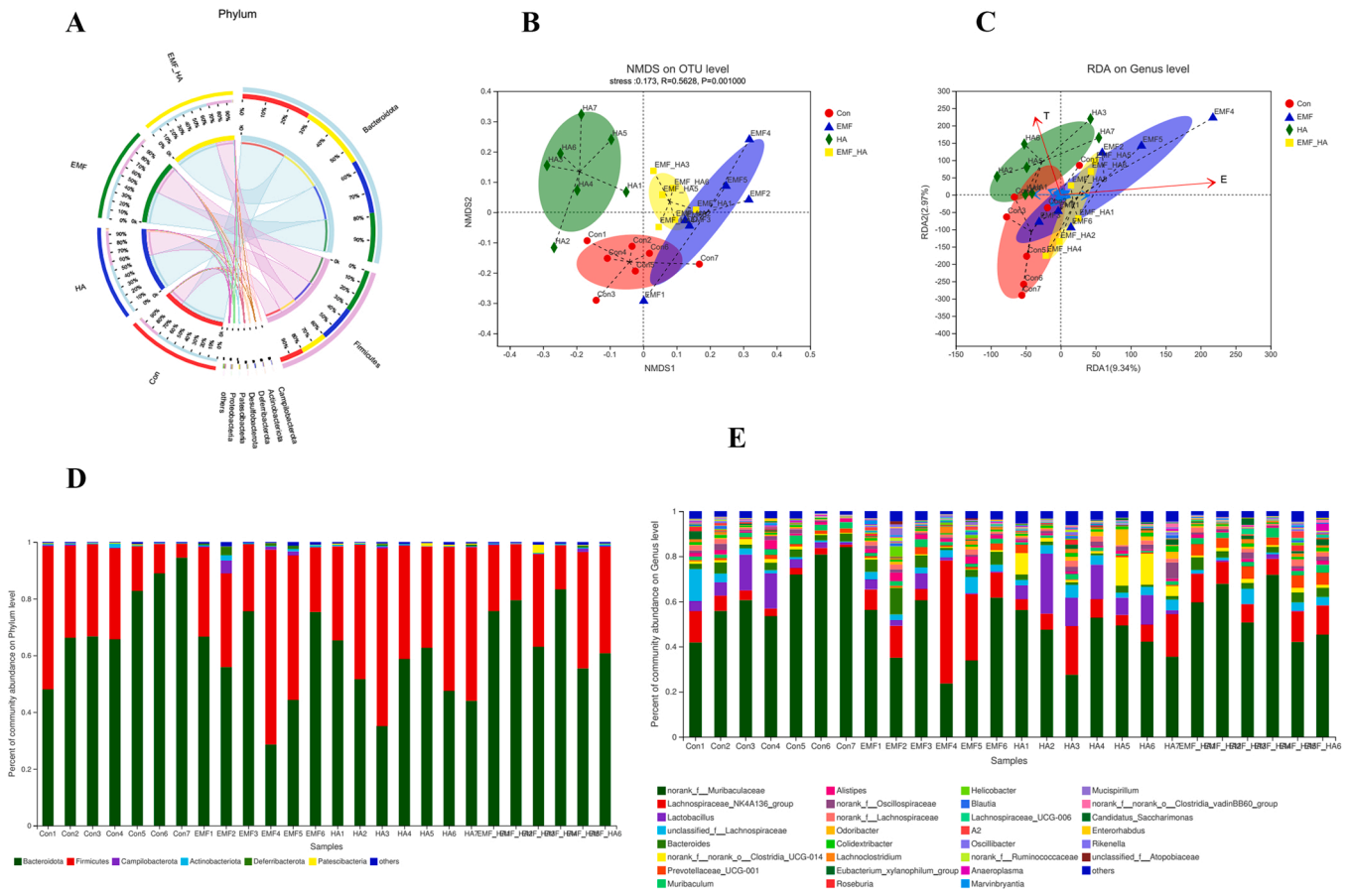


Fig. 4. Community composition of the gut microbiota. A. Relative abundance at the phylum level in each group. B. Beta diversity assessed by Nonmetric Multidimensional Scaling (NMDS), based on Bray-Curtis. Anosim tests show statistically significant differences among these groups. C. Redundancy analysis (RDA) of gut microbiota (symbols) and environmental characteristics (arrows). D. Relative community abundance at the phylum level in each sample. E. Relative community abundance at the genus level in each sample.

0.1413), *g_Odoribacter* ($p = 0.03832$, *corrected p-value* $= 0.1413$), and *g_Colidextribacter* ($p = 0.008133$, *corrected p-value* $= 0.1066$) were significantly increased, while *g_Alistipes* ($p = 0.003405$, *corrected p-value* $= 0.0893$) was significantly decreased in the EMF+HA group compared with the EMF group (Fig. 5C).

3.5. HA alleviated depression-related serum metabolite disturbance caused by EMF exposure

To identify the differential metabolites between the EMF and EMF+HA groups, we calculated fold changes (FCs), *P* values and PLS-DA variable importance in the projection (VIP) scores for all metabolic features in EMF vs. EMF+HA. In total, 56 significant metabolic features (FC $\geq$ 2 or $\leq$ 0.5, P $\leq$ 0.05, and VIP $>$ 1) were found (Table 2). Compared with EMF exposure, HA balanced serum metabolite disturbance (Fig. 6A). These metabolites were classified as Kyoto Encyclopedia of Genes and Genomes (KEGG) compounds. The hormone and transmitter groups included 4 kinds of neurotransmitters and 2 kinds of steroid hormones. Lipids involved were eicosanoids and fatty acids. Among them, there was 1 eicosanoid and 16 fatty acids. Organic acids are carboxylic acids and there were 18 such compounds in the analysis. The peptides included amines, amino acids and peptides. Among them, there were 3 kinds of amine compounds, 23 kinds of amino acid compounds and 2 kinds of peptide compounds (Tables 1, 2).

To further understand these metabolic changes at the pathway level, we performed pathway enrichment analysis. The following metabolic pathways were significantly changed: Metabolic pathways (map01100, Number of metabolite: 42); ABC transporters (map02010, Number of metabolite: 8); Protein digestion and absorption (map04974, Number of metabolite: 5); Vitamin digestion and absorption (map04977, Number of metabolite: 5); Tryptophan metabolism (map00380, Number of metabolite: 4); Central carbon metabolism in cancer (map05230, Number of metabolite: 4); Fructose and mannose metabolism (map00051, Number of metabolite: 3); Pyrimidine metabolism (map00240, Number of metabolite: 3); Cysteine and methionine metabolism (map00270, Number of metabolite: 3); Histidine metabolism (map00340, Number of metabolite: 3); Tyrosine metabolism (map00350, Number of metabolite: 3); Phenylalanine metabolism (map00360, Number of metabolite: 3); Metabolism of xenobiotics by cytochrome P450 (map00980, Number of metabolite: 3); Carbon metabolite (map01200, Number of metabolite: 3); Biosynthesis of amino acids (map01230, Number of metabolite: 3) and other related pathways (Fig. 6C).

The markedly different metabolic pathways ($Q < 0.05$) were analyzed with PICRUSt and include the following: energy production and conversion, carbohydrate transport and metabolism, amino acid transport and metabolism, nucleotide transport and metabolism, lipid transport and metabolism, inorganic ion transport and metabolism (Fig. 6B). Both the metabolic pathway analysis of differential metabolites and the PICRUSt functional prediction of gut microbiota results highlighted tryptophan metabolism, pyrimidine metabolism, cysteine and methionine metabolism, histidine metabolism, tyrosine metabolism and phenylalanine acid metabolism, amino acid biosynthesis and other related pathways. This result suggested that changes in these metabolic pathways may be an important way in which HA improves depression-like behaviors caused by EMF exposure and that the gut microbiota plays an essential role.

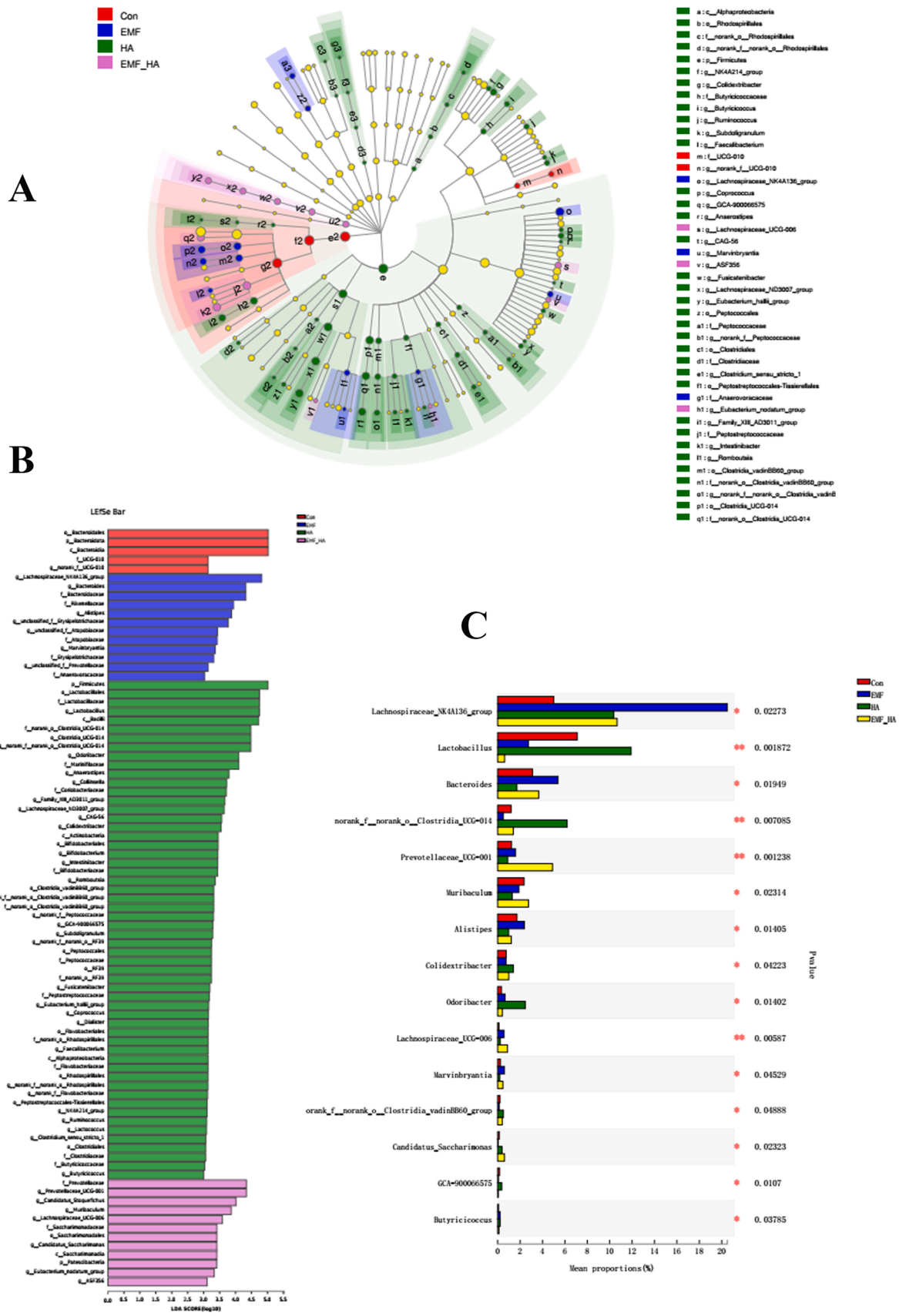


Fig. 5. The composition difference of gut microbiota in each group. A. The composition cladogram with microbial differences in each group. B. Differential bacterial taxonomy selected by LEfSe analysis with LDA score > 2 in microbiota. C. Differentiated gut microbiome in each group.

Table 1
List of significantly changed metabolites in the EMF group.

	mean_Con	sd_Con	mean_EMF	sd_EMF	Fold Change	log2 (FC)	*p*.value	FDR
Ketoleucine	13557745	9987354	42109179	9537258	0.322	-1.635	0.000275	0.0764
Formylanthranilic acid	1060665	225981.3	2230218	610776.3	0.476	-1.072	0.000618	0.0859
D-Fructose	7438820	2747047	29549500	15186521	0.252	-1.99	0.00291	0.269
Dodecanoic acid	1.22E+08	30865425	1.95E+08	43748231	0.629	-0.669	0.00512	0.356
Cholesterol	205.492	0	3.67E+08	3.04E+08	0	-20.767	0.00826	0.428
Gluconic acid	4.91E+08	1.04E+08	3.3E+08	86494554	1.489	0.574	0.0119	0.428
Phenylacetic acid	4336659	1443703	6517230	1183502	0.665	-0.588	0.0134	0.428
Sedoheptulose 7-phosphate	2293697	703223.3	3551024	859243.1	0.646	-0.631	0.0143	0.428
Maleic acid	1.44E+08	43019261	3E+08	1.36E+08	0.479	-1.061	0.0146	0.428
Fumaric acid	3976940	1435102	2105986	748998.8	1.888	0.917	0.0154	0.428
D-Mannose	5409433	517759.6	6458740	831366.2	0.838	-0.256	0.0179	0.451
Pimelic acid	1.39E+08	8320126	1.5E+08	4905277	0.929	-0.106	0.0195	0.451
2-Dehydropantoate	8187159	1024773	6864637	741629	1.193	0.254	0.0238	0.509
4-Hydroxycinnamic acid	82988153	71290206	3.15E+08	2.42E+08	0.263	-1.927	0.033	0.53
Phenylacetylglycine	41457716	13219368	79187282	39815664	0.524	-0.934	0.0369	0.53
N-Formyl-L-glutamic acid	4688155	1287652	6814099	1942447	0.688	-0.54	0.0377	0.53
Imidazole-4-acetaldehyde	8653720	1207952	6375274	2228077	1.357	0.441	0.0389	0.53
Oxalacetic acid	1.02E+08	39997079	62312192	9522185	1.631	0.706	0.0394	0.53
Quercetin	46502840	2510558	51005127	4429505	0.912	-0.133	0.0419	0.53
(R)-3-Hydroxybutyric acid	36030298	15803514	50875887	3442919	0.708	-0.498	0.0465	0.53
1-Methylxanthine	1318483	117432.8	1097146	230844.3	1.202	0.265	0.0473	0.53
Lathosterol	99675324	60531305	3.15E+08	2.51E+08	0.316	-1.662	0.0487	0.53

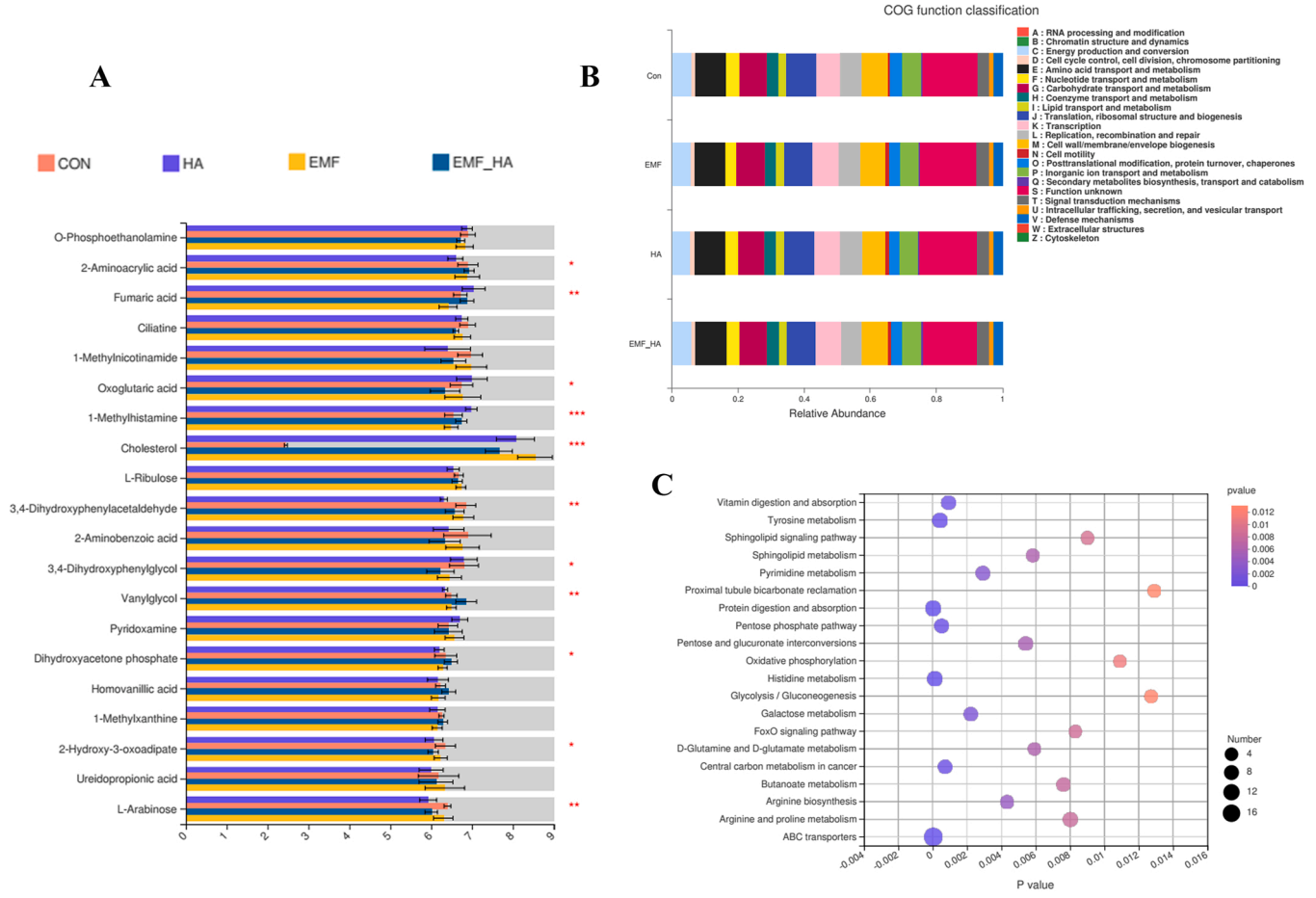


Fig. 6. Differential serum metabolites. A. The Kruskal-Wallis H test of differential serum metabolites. B. Prediction of serum metabolite function with PICRUSt. C. KEGG pathway enrichment analysis.

Table 2
List of significantly changed metabolites in the EMF with HA group.

	mean_EMF	sd_EMF	mean_EMF+HA	sd_EMF+HA	Fold Change	log2 (FC)	*p*.value	FDR	VIP
Ketoleucine	3.15E+08	2.51E+08	22499470	20838811	14.017	3.809	0.0173	0.171	1.566
Putrescine	3.67E+08	3.04E+08	40404925	50035431	9.077	3.182	0.0269	0.191	1.483
2-Dehydropantoate	42109179	9537258	5448375	5481740	7.729	2.95	9.86E-06	0.00274	2.181
Thymidine	40458846	28798503	6037831	2374788	6.701	2.744	0.0154	0.164	1.586
Eicosadienoic acid	46970616	33948237	8995730	4033056	5.221	2.384	0.0215	0.191	1.525
4-Oxoproline	9701122	6380189	2626945	1936913	3.693	1.885	0.0265	0.191	1.485
Guanidoacetic acid	2.84E+08	2E+08	77066743	58174506	3.688	1.883	0.0353	0.202	1.426
Se-Methylselenocysteine	3.43E+08	2.09E+08	96155905	1.12E+08	3.57	1.836	0.0286	0.192	1.47
Methyl beta-D-galactoside	1687451	795802	635411.2	194344.6	2.656	1.409	0.0104	0.147	1.649
2-Keto-6-aminocaproate	21348132	11432262	8619585	1509186	2.477	1.308	0.0222	0.191	1.52
L-Kynurenine	24448917	12510853	11058313	2259951	2.211	1.145	0.0274	0.191	1.478
Niacinamide	54441030	26016108	25079263	12984822	2.171	1.118	0.0329	0.199	1.441
Folic acid	4.88E+08	2.4E+08	2.28E+08	42018380	2.143	1.099	0.026	0.191	1.489
N-Carbamoylputrescine	5387896	2550829	2521743	1359418	2.137	1.095	0.0355	0.202	1.425
4-Guanidinobutanal	13839849	2462334	7057165	3035022	1.961	0.972	0.00169	0.0938	1.876
Cytidine	1377753	449631.9	712025.4	245448	1.935	0.952	0.00976	0.147	1.659
2-Hydroxy-3-oxoadipate	3E+08	1.36E+08	1.55E+08	64794517	1.932	0.95	0.0404	0.218	1.396
L-Arabinose	65129673	12166214	37213701	13348391	1.75	0.807	0.00357	0.115	1.795
N-Formyl-L-methionine	1.63E+08	67209803	93652299	22805217	1.745	0.803	0.0368	0.205	1.417
D-Ribose	71996977	22522340	44191264	11552218	1.629	0.704	0.0227	0.191	1.516
Fumaric acid	18900531	2273132	11732775	852645	1.611	0.688	2.82E-05	0.00392	2.143
D-Mannose	1.69E+10	3.65E+09	1.05E+10	5.75E+09	1.607	0.684	0.0441	0.219	1.377
Hydrogen phosphate	12767309	3903506	7983098	2324926	1.599	0.677	0.0274	0.191	1.478
Sucrose	15545286	2399047	9826436	1733432	1.582	0.662	0.000801	0.0556	1.945
Palmitic acid	27529590	7928625	17579666	7004914	1.566	0.647	0.044	0.219	1.377
Inosine	3.36E+08	75144817	2.17E+08	63989019	1.548	0.63	0.0144	0.16	1.597
3-Hydroxyphenylacetic acid	2283694	200400.5	1505173	633303.3	1.517	0.601	0.0166	0.171	1.572
Lathosterol	52099839	15188176	34790758	10354949	1.498	0.583	0.0438	0.219	1.378
UMP	3551024	859243.1	2373671	559849	1.496	0.581	0.0184	0.171	1.554
Sedoheptulose 7-phosphate	10939302	1133576	7827837	2910869	1.397	0.483	0.0349	0.202	1.429
2-Naphthol	21169437	3179682	15803033	942038.5	1.34	0.422	0.00267	0.115	1.828
Guanosine	58177826	8563157	44352192	11692339	1.312	0.391	0.0416	0.218	1.39
Adenosine	23831609	4779105	18183600	2847463	1.311	0.39	0.0322	0.199	1.446
L-Carnitine	12244273	1664254	9384327	1173027	1.305	0.384	0.00633	0.126	1.722
Acetylphosphate	3.43E+08	63240912	2.63E+08	54761929	1.304	0.383	0.0408	0.218	1.394
Indolepyruvate	92445158	11648876	72398594	9294820	1.277	0.353	0.00808	0.14	1.687
1-Methylnicotinamide	16228129	2964455	12780440	1301690	1.27	0.345	0.0261	0.191	1.488
Cholesterol	50490032	8482762	39758450	6148101	1.27	0.345	0.031	0.196	1.454
Uric acid	7059540	932662.2	5565286	900114.7	1.268	0.343	0.018	0.171	1.558
GMP	92901461	15309906	73397248	5042699	1.266	0.34	0.0142	0.16	1.599
Butyric acid	68166162	7702257	55443384	6496494	1.23	0.298	0.0114	0.147	1.635
Phthalate	6458740	831366.2	5265719	471670.4	1.227	0.295	0.0121	0.147	1.626
3-Dehydroshikimate	14919402	487154.1	13062847	1120240	1.142	0.192	0.00396	0.115	1.782
Pseudouridine	6.99E+09	7.25E+08	6.16E+09	2.3E+08	1.134	0.182	0.0236	0.191	1.508
(R) 2,3-Dihydroxy-3-methylvalerate	8301519	156105.6	8931539	585565	0.929	-0.106	0.029	0.192	1.467
N-Acetyldemethylphosphinothricin	7.55E+08	2.17E+08	1.08E+09	57775876	0.697	-0.521	0.00495	0.115	1.754
CMP	6864637	741629	10098170	1077362	0.68	-0.557	0.000123	0.0114	2.073
Quinolinic acid	2586033	1207668	4792935	1236533	0.54	-0.89	0.0107	0.147	1.645
3,4-Dihydroxyphenylacetaldehyde	688405.7	306227.6	1565816	633233.5	0.44	-1.186	0.0121	0.147	1.625
Saccharopine	2105986	748998.8	4892561	2078362	0.43	-1.216	0.0115	0.147	1.634
Maleic acid	5020768	2269353	15262667	7194074	0.329	-1.604	0.00767	0.14	1.695
L-Histidine	67750704	26377973	2.08E+08	87563726	0.326	-1.617	0.00378	0.115	1.788
all-trans-Retinoic acid	2977815	1700889	9817480	4266513	0.303	-1.721	0.00448	0.115	1.767
Citric acid	1656431	447281.5	5653697	2687767	0.293	-1.771	0.0049	0.115	1.756
Pyrrolidonecarboxylic acid	11538653	9665587	49547858	25283399	0.233	-2.102	0.00634	0.126	1.722
Dehydroepiandrosterone	15965241	13747287	82655531	63204907	0.193	-2.372	0.0301	0.195	1.459

4. Discussion

Our living environment has been increasingly polluted by EMFs, while the central nervous system is vulnerable to these adverse effects. Epidemiological studies suggest that long-term EMF exposure could cause sleep disorders, headaches, fatigue, memory loss (Jiang et al., 2015). EMF exposure could even lead to cognitive behavior disorders, such as depression and anxiety (Genuis, 2008). It was not clear whether EMFs, as a physical environmental factor, could cause central nervous system dysfunction in experimental animals. In our study, after 5 weeks of continuous intermittent EMF exposure, we found that the mice had depression-like neurobehavioral changes (Fig. 2). Additionally, 16S rRNA gene sequencing was used to reveal the community composition of the gut microbiota. EMF exposure significantly influenced the beta diversity instead of alpha diversity of the gut microbiota.

The correlation between the gut microbiota and depression has been scientifically confirmed (Collins and Bercik, 2009; Kleiman et al., 2017). The community composition of the gut microbiota in depressed patients or animals is significantly different from that in regular control groups. Fecal microbiota transplantation (FMT) from donors of depression patients can even induce depression-like behavior in sterile rats. This further proves that gut microbiota and depression have a direct relationship. In Jiajia Duan's research, depressed animals exhibited gut microbiomes that were depleted in members of the family *Lactobacillaceae* (Jiajia et al., 2021). In our study, EMF exposure not only caused depression-like neurobehavioral disorders but also significantly depleted *g_Lactobacillus*. We speculated that an imbalanced composition of the gut microbiota with EMF exposure might be one of the most

important factors for depression-like symptom occurrence.

It has not been previously reported that HA can alleviate central nervous system dysfunction associated with EMF exposure. In our study, HA alleviated depression-like factors (Fig. 3) and the EMF exposure lasted 5 weeks. To maintain the community composition of the gut microbiota established by HA, we continued to maintain the HA environment after daily EMF exposure.

Both EMF and HA regulated the ratio of *p_Firmicutes* and *p_Bacteroidota*. LEfSe was carried out to identify differentially abundant bacterial taxa among all groups. EMF exposure caused the proportion of 6 kinds of bacteria to change significantly, including those of *g_Butyricicoccus* and *g_Anaerotruncus*. HA restored the imbalance of gut microbes caused by EMF exposure, and the proportion of probiotics (*g_Lactobacillus*) increased significantly. RDA revealed that as an environmental characteristic, the effect of EMF was more significant than that of temperature. Yang et al. found that long-term exposure to high environmental temperature could affect the gut microbiome in mice. In their study, genus-level analysis showed that the genus Lactobacillus (a major probiotic) was significantly increased, which was consistent with our findings. *Bacillus subtilis* has been used for the prevention of heat stress by maintaining intestinal permeability and microbial structure, as well as reducing bacterial translocation (Moore et al., 2014; Sorokulova et al., 2016). In our study, *Bacillus subtilis* was not found, but *Clostridia* was found to be increased.

Growing evidence indicates that the gut microbiota affects not only gastrointestinal function but also central nervous system (CNS) physiology and behavior by regulating the microbiota-gut-brain axis (Evrensel and Ceylan, 2015; Foster and Mcvey Neufeld, 2013; Leclercq et al., 2020; Luna and Foster, 2015; Luna et al., 2015). Concerning the metabolome, existing data on depression have uncovered altered metabolic pathways and metabolites in urine, plasma and hippocampal samples from clinical subjects and animal models (Su et al., 2011, 2014; Zheng et al., 2012). Several metabolites that have been shown to be altered with depression include hippuric acid, tryptophan, phenylalanine (Machado et al., 2012; Bansal et al., 2018). These are metabolic byproducts of the gut microbiota. In our study, EMF induced disturbance in the metabolite profiles of serum samples. Significantly different metabolites included cholesterol, D-fructose and fumaric acid and these were associated with depression (Xiong et al., 2020). Based on KEGG classification, the metabolites involved in neurotransmitters and steroids were altered significantly. HA alleviated depression-related serum metabolite disturbance caused by EMF exposure. Metabolic pathways involved in HA were protein digestion and absorption, vitamin digestion and absorption, tryptophan metabolism, pyrimidine metabolism, tyrosine metabolism, phenylalanine metabolism, amino acid biosynthesis. Valine, leucine and isoleucine are essential branched-chain amino acids, and their biosynthetic pathways play an important role in the stress response and energy metabolism balance. Both the metabolic pathway analysis of differential metabolites and the PICRUSt functional prediction of gut microbiota pointed to tryptophan metabolism, pyrimidine metabolism, cysteine and methionine metabolism, histidine metabolism, tyrosine metabolism and phenylalanine acid metabolism, amino acid biosynthesis and other related pathways as being important. This result suggested that changes in these metabolic pathways may be an important factor in the improvement of depression-like behaviors caused by EMF via HA and that the gut microbiota plays an essential role.

This study had limitations. To characterize the effects of HA on depression-like disorders linked to the gut microbiota, fecal and serum samples were collected and sequenced only at the end of the experiment. However, both EMF exposure and HA were long-term chronic processes. The gut microbiomes and serum metabolites might have been explored at additional time points for more reliable and precise results using both comparative and longitudinal analyses. Although 16S rRNA gene sequencing technology was beneficial for microbiome studies, it provided low resolution at the species level and insufficient discriminatory power for some genera. Ideally, whole-metagenome shotgun sequencing would be a better choice for obtaining more accurate information. Although we found depression-related gut microbiota and serum metabolites, exploration of the correlation between the gut microbiota and serum metabolites was lacking and should be performed in the future. The findings in this study were correlational and did not support a causal relationship between gut microbiota and EMF exposure or HA, indicating a lack of sufficient evidence to distinguish the gut microbiota as a cause or consequence of the effects following EMF exposure or HA. Future studies using strategies such as fecal microbiota transplantation (FMT) are necessary to elucidate the underlying causality.

Nevertheless, our study demonstrated that EMF exposure could not only lead to neurobehavioral disorders such as depression, but also cause gut microbiota imbalance. HA alleviated the depression features caused by EMF exposure, providing an example of cross-tolerance. Based on the analysis between gut microbiota and serum metabolites, we speculated that gut microbiota might play an important role in the cross-tolerance of HA.

5. Conclusion

Depression- and anxiety-like behaviors were detected by behavioral experiments. EMF exposure could lead to depression-like neurobehavioral disorders. HA alleviated the depression features caused by EMF exposure. Both EMF and HA regulated the ratio of *p_Firmicutes* and *p_Bacteroidota*. EMF exposure caused the proportion of 6 kinds of bacteria, such as *g_Butyricicoccus* and *g_Anaerotruncus,* changing significantly. HA restored the imbalance of gut microbes caused by EMF exposure and the proportion of probiotics (*g_Lactobacillus*) increased significantly. Serum metabolite analysis suggested that HA alleviated the disturbance of serum metabolites (such as cholesterol and D-mannose) induced by EMF exposure. Both the metabolic KEGG pathways and PICRUSt functional analysis indicated that tryptophan metabolism, pyrimidine metabolism and amino acid biosynthesis were involved.

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Author statement

Manuscript by Xueyan Huang, Zhen Luo, Zeze Wang, Genlin He, Yulong Tan, Boyi Zhang, Huan Zhou, Ping Li, Tingting Shen, Xueting Yu, Xue Luo, Xuesen Yang and entitled “Electromagnetic fields exposure induced depression features could be alleviated by heat acclimation base on remodeling gut microbiota”, which is submitted for possible publication in *Environmental Pollution.* This paper is new. Neither the entire paper nor any part of its content has been published or has been accepted elsewhere. It is not being submitted to any other journal.

Code availability

Not applicable.

Authors’ contributions

The Author X.L. and XS.Y. conceived the study, designed and performed the experiments, analyzed the data and wrote the manuscript; Z. L., GL.H., ZZ.W., YL.T. and XY.H. performed experiments, and analyzed the data; YL.T., BY.Z., H.Z., P.L., TT.S., ZZ. W., and XT.Y. were involved in data generation. X.L. conceived the study and revised the manuscript. XS.Y. supervised experimental design and revised the manuscript. All authors reviewed the manuscript.

Ethics approval

The animal study was reviewed and approved by the Experimental Animal Ethics Committee of Army Medical University.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

The raw sequences used in this study were stored on the Sequence Read Archive (SRA) of NCBI, and the SRA accession number was SRP329366. Bioproject accession number was PRJNA748853.

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Update

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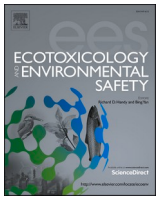
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Corrigendum



Corrigendum to "Electromagnetic field exposure-induced depression features could be alleviated by heat acclimation based on remodeling the gut microbiota," [Ecotoxicol. Environ. Saf., 228 (2021) 112980]

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The author of this article regrets the inappropriate expression of references in the manuscript. Based on feedback from readers and us re proofreading of the entire text, we have revised the manuscript. We have presented the revised details as follows. The relevant modifications are highlighted in red font.

The authors would like to apologise for any inconvenience caused.

1. **Original text:**

The impact of electromagnetic pollution on health has attracted public attention. EMF regulation (2016) clearly noted that long-term low-dose electromagnetic radiation exposure can lead to central nervous system dysfunction (Van, 2000; Wijngaarden, 2000).

References

Wijngaarden, E.V. 2000. Exposure to electromagnetic fields and suicide among electric utility workers: a nested case-control study.

Revised version:

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References

van Wijngaarden E, Savitz DA, Kleckner RC, Cai J, Loomis D. Exposure to electromagnetic fields and suicide among electric utility workers: a nested case-control study. West J Med. 2000 Aug;173 (2):94–100. doi: 10.1136/ewjm.173.2.94. PMID: 10924428; PMCID: PMC1071010.

2. **Original text:**

In 2020, Yee. et al. found that the gut microbiome ratio of Firmicutes/Bacteroidetes decreased with pulsing electromagnetic field (PEMF) exposure (Yee et al., 2020).

References

Tai, Y.K., Ng, C., K, Yap, J.L.Y., Yin, J.N., Wong, C., Patel, B.K., Soong, P.L., Pelczar, P., Frohlich, J., Beyer, C., Fong, C.H.H., Ramanan, S., Casarosa, M., Cerrato, C.P., Foo, Z.L., Pannir Selvan, R.M., Grishina,

1. Introduction

Electromagnetic fields (EMFs) are widely used in communication, smelting, medicine and other industries. The impact of electromagnetic pollution on health has attracted public attention. EMF regulation (2016) clearly noted that long-term low-dose electromagnetic radiation exposure can lead to central nervous system dysfunction (Van, 2000; Wijngaarden, 2000). EMF exposure can cause neurobehavioral disorders, including circadian imbalance, headache, fatigue, depression and anxiety. However, it is difficult to verify the conclusions of epidemiological studies on the mechanism of nerve injury induced by EMF exposure, especially low-dose EMF exposure. Some of the obtained results have even been contradictory. One reason might be that

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system disorders by affecting gut microbes. Currently, there are few reports about the bioeffects of gut microbes under EMF exposure. In 2020, Yee. et al. found that the gut microbiome ratio of *Firmicutes/Bacteroidetes* decreased with pulsing electromagnetic field (PEMF) exposure (Yee et al., 2020). Therefore, EMF exposure could be able to affect the gut microbiota composition.

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References

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Our previous study also revealed that HA could regulate microglial inflammatory activation with EMF exposure (Genlin et al., 2020).

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tolerance". Our previous study also revealed that HA could regulate microglial inflammatory activation with EMF exposure (Genlin et al., 2020). Moreover, long-term exposure to high environmental temperature has been shown to affect the gut microbiome in mice (Yang et al., 2021). According to the "cross tolerance" of HA, we hypothesize that HA

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4. **Original text:**

Moreover, long-term exposure to high environmental temperature has been shown to affect the gut microbiome in mice (Yang et al., 2021).

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5. Original text:

In Jiajia Duan's research, depressed animals exhibited gut microbiomes that were depleted in members of the family Lactobacillaceae (Jiajia et al., 2021).

References

Duan, J., Huang, Y., Tan, X., Chai, T., Wu, J., Zhang, H., Li, Y., Hu, X., Zheng, P., Ji, P., Zhao, L., Yang, D., Fang, L., Song, J., Xie, P., 2021. Characterization of gut microbiome in mice model of depression with divergent response to escitalopram treatment. Transl. Psychiatry 11 (1), 303.

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References

Luna, R.A., Foster, J.A., 2015. Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression. Curr. Opin. Biotechnol. 32,

35–41.

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